Limnologica 57 (2016) 66–72

Contents lists available at ScienceDirect

Limnologica

journal homepage: www.elsevier.com/locate/limno

Food utilization of shell-attached algae contributes to the growth of host mud snail, *Bellamya chinensis*: Evidence from fatty acid biomarkers and carbon stable isotope analysis

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ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form 6 November 2015
Accepted 25 November 2015
Available online 11 January 2016

Keywords:
Shell fouling
Food quality
Cipangopaludina
Food web analysis
Mud snail

ABSTRACT

Dense algal growth on shells of the freshwater mud snail species, *Bellamya chinensis*, is commonly found. In rice paddy fields of Northeastern Japan, fatty acid biomarkers and carbon stable isotope composition were analyzed to test whether *B. chinensis* grazes on shell-attached algae. The carbon stable isotope ratio of *B. chinensis* was positively related to that of shell-attached algae. *B. chinensis* also assimilated substantial amounts of omega-6 fatty acids, which were abundant in shell-attached algae. Furthermore, the effect of assimilating shell-attached algae on *B. chinensis* growth was examined in a field experiment. Individuals feeding on shell-attached algae exhibited faster shell growth than those with no access to shell-attached algae of other individuals. Our results demonstrate that *B. chinensis* growth is enhanced by algal fouling on their own shells, which provides them with a nutritious food source, although very few studies have documented benefits conferred to an organism that hosts an epibiotic species.

Introduction

Aquatic mollusk shells serve as hard substrate for other organisms such as algae, sponges, polychaetes, anthozoans, and other mollusks (Gutierrea et al., 2003). Such epibiotic organisms have been reported to detrimentally affect host species' growth rate and fitness (Wahl, 1996; Chan and Chan, 2005; Sievers et al., 2013). Therefore, some bivalve species possess anti-biofouling mechanisms, such as chemical defenses (Becker, 1995; Bers et al., 2006) and an anti-fouling shell structure (Scardino et al., 2003; Bers et al., 2010). However, the potential advantages of biofouling to the host species have also been suggested. For instance, Abbott and Bergey (2007) showed that epibiotic algae on snail shells were a food source for other snail individuals. However, this feeding behavior has not been reported in the field and the degree of nutritional contribution of shell-attached algae to host species is unknown.

Bellamya (=*Cipangopaludina*) *chinensis* (Gray, 1834) is one of the most common freshwater snails in Japanese freshwater ecosystems including paddy fields. Although biofouling is rarely found in other Japanese freshwater snail species, *B. chinensis* is known to be densely fouled by shell-attached algae which mainly consist of filamentous algal species (Fig. 1). *B. chinensis* have taenioglossid radulae as feeding apparatus (Jokinen, 1982) which is appropriate for feeding on filamentous algae (Steneck and Watling, 1982). Thus, shell-attached algae would be suitable food sources for *B. chinensis*. Moreover, in paddy fields predominantly covered by soft sediment, snail shells seems to provide rare hard substrates suitable for filamentous algae which may valuable resource for snail species. Jokinen (1982) reported that epiphytic and benthic algae were detected in gut contents of *B. chinensis* collected from river, canal and lake ecosystems. However, the origin of epiphytic algae consumed by *B. chinensis* or importance of shell-attached algae for this species in systems predominated by soft sediment remains unknown. While *B. chinensis* cannot access their own shell, we hypothesize that *B. chinensis* graze attached algae growing on shells of conspecific individuals in rice paddy fields with little hard substrates.

To examine food webs, gut content analyses have been traditionally applied in ecology. However, gut content analysis is influenced by differences in digestion resistance among food sources and tends to reflect the materials that were grazed immediately before sample collection (Pasquaud et al., 2007) rather than those that were consumed over the long term. Therefore, stable isotope analysis has recently become widely used in snail food web studies (e.g. Kurata et al., 2001; Lau et al., 2009) and has been used to examine the relationship between gastropod species and their epibiotic organisms (Zabala et al., 2013). Stable carbon

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http://dx.doi.org/10.1016/j.limno.2015.11.006
0075-9511/

Fig. 1. Shell surface of mud snail, *Bellamya chinensis*, which was collected from a paddy field of Shichigahama area.

isotope ratios in animals reflect the signatures of their assimilated food sources (Pasquaud et al., 2007). Fatty acids are also used to study trophic exchanges, including revealing assimilated food sources for snails (Alfaro et al., 2007). This is because fatty acids are conservatively transferred to higher trophic levels and some fatty acids are taxon-specific (Pasquaud et al., 2007). Both stable isotope and fatty acid methods provide time-integrated information on animal food sources. Although these methods have not been used to determine *B. chinensis* food sources, they have the potential to be powerful tools to evaluate the snail's assimilation of shell-attached algae.

The objectives of this study were to test: (1) whether *B. chinensis* uses shell-attached algae as a food source, and (2) whether the assimilation of shell-attached algae enhances host snail growth. Both field observations and a field experiment were conducted in rice paddy systems, employing fatty acid biomarker and stable carbon isotope analyses.

Materials and methods

Field surveys

We investigated rice paddy fields in the Miyagi prefecture of northeastern Japan (38.2957°N, 140.8563°E). Samples were collected in July and September 2007, July and August 2008, and August 2009 from the Shichigahama area of Miyagi (38.2935°N, 141.0522°E) and in June 2011 from the Obari area of Miyagi (38.1751°N, 140.6728°E) (Fig. 2). The sampled fields were different each time. Samples were collected from both ends and the middle of one side of each rice paddy for three sampling locations per field.

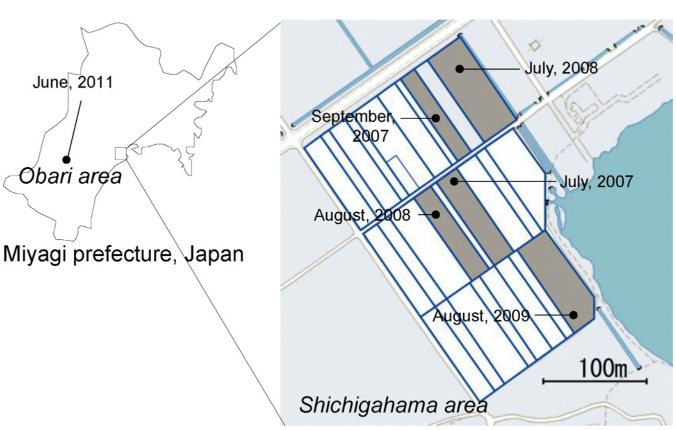

Fig. 2. Location of field survey sites of six paddy fields in Miyagi prefecture, Japan.

Five individuals of *B. chinensis* with a shell length of ca. 3 cm were sampled at each of the three field locations. One of these five individuals was randomly selected and kept in a tank filled with water for 24 h to allow the snail to eject its gut contents. This individual was then dissected to obtain soft tissue for later analysis of stable carbon isotopes and fatty acid composition. In this procedure, 3 samples were prepared for analyses for stable carbon isotope, carbon and nitrogen contents, and fatty acid composition in each study site. The analyses for elements and fatty acid analyses were conducted for samples from July, 2008.

To analyze for the chemical compositions of potential food sources of *B. chinensis*, rice paddy sediment organic matter (SOM) and *B. chinensis* shell-attached algae were sampled. A sample containing the top 5 mm of sediment as well as the surrounding water was obtained in the form of slurry by using a syringe in the submerged paddy fields. *B. chinensis* feces on the sediment surface were avoided during samplings. In the laboratory, the slurry samples were poured into a plastic bottle and allowed to settle for 2 h. Sediment deposits were retrieved for the later analyses. Algae attached on shells of the five *B. chinensis* individuals were scraped off by a brush and rinsed into distilled water. The slurries containing shell-attached algae were filtered through glass fiber filters (Whatman, GF/F) and particle samples on the glass filters were analyzed. All samples were preserved in a freezer at −30 °C until analysis and analyzed within 1 month, since we previously confirmed that preservation for <1 month did not affect fatty acid composition of paddy field sediments.

All samples were analyzed for carbon stable isotope ratios. Only samples collected in July 2008 were analyzed for fatty acid composition, and carbon and nitrogen contents.

Field experiment

To examine the effect of consumption of shell-attached algae by *B. chinensis* on their growth, a field experiment was conducted from August to September in 2009. An artificial pond was built in a dry fallow field in Miyagi prefecture. The surface area and water depth of this artificial pond were 22.7 m^2 (8.4 m × 2.7 m) in area and 0.1 m in depth. The experimental pond was filled with water irrigated from an adjacent channel and colonized by no native *B. chinensis*.

B. chinensis with approximately 3-cm in shell length were selectively collected from one of the field investigation sites of August 2009 where *B. chinensis* recorded the highest density among our study sites and enabled to easily collect sufficient number of individuals for the later experiment. The shell length of all *B. chinensis* individuals collected were measured by using a vernier caliper. After labeling individuals with white paint, 220 snails were released into the experimental pond. Eight cages made from stainless steel wire nets (5 mm mesh size, 0.33 m wide × 0.30 m deep × 0.30 m high) were placed in the artificial experimental pond, and one individual of *B. chinensis* was installed in each cage. The density of *B. chinensis* both outside and inside of the cages was almost equivalent (i.e., 1 individual 0.1 m^{-2}). Since *B. chinensis* cannot graze shell-attached algae on their own shell, the *B. chinensis* individuals that were released alone in the cages had no chance to graze shell-attached algae. *B. chinensis* released outside of the cages could access shell-attached algae on other individuals. These snails were designated as 'excluded' and 'caged' snails, respectively.

At 1- and 2-month after starting the experiment, the eight individuals of caged snails were sampled, and eight excluded individuals were also randomly collected. Those snails were measured for their shell length. At 2-month later, SOM was also sampled from the inside of each cage. Shell-attached algae were obtained from